Invertebrate Biology 118(3): 221–235.

The choanosome of hexactinellid sponges

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Abstract. Three-dimensional images of the choanosome of hexactinellid sponges can help in explaining how these sponges mix cellular and syncytial tissues in a fine cobweb-like network. Plastic replicas of the water canals show that *Rhabdocalyptus dawsoni* has large, highly branched incurrent canals, and equally large, although less branched, excurrent canals that are studded with 60 μm-long flagellated chambers. Freeze-fractured, fixed specimens show the syncytial tissue, known as the trabecular reticulum, envelops cells in a thin collagenous mesohyl at the flagellated chambers in both *R. dawsoni* and *Aphrocallistes vastus*. Groups of archaeocytes are especially abundant in the mesohyl of *R. dawsoni* during summer months. A branch of the trabecular reticulum, the secondary reticulum, surrounds the collars in the flagellated chambers, effectively forcing water to be drawn through the collar microvilli. Another branch, the inner membrane, occupies up to 10% of flagellated chambers of sponges during all months of the year, but is especially prevalent in specimens which have been kept in sea water aquaria for several weeks. This three-dimensional view of hexactinellid tissues reinforces the conclusion that hexactinellid sponges be separated from other sponges at the subphylum level.

Additional key words: Hexactinellida, Porifera, syncytium, sponge

Sponge morphology has been studied for well over a century. These animals are considered to be simple, radially symmetric or asymmetric, diploblastic metazoans that carry out all the basic functions of ingestion and egestion, growth and reproduction with a very small complement of cell types. The Hexactinellida, however, differ fundamentally from other sponges in having a syncytial tissue (the trabecular reticulum) rather than cells, as their major tissue component (Reiswig 1979; Mackie & Singla 1983; Leys 1995).

The uniqueness of the construction of the Hexactinellida has been recognized since these animals were first examined in the late 1800s. Bidder (1929) first proposed separating the Porifera into two phyla based partly on the difference in tissue organization between cellular and syncytial sponges. Later, Reid (1963) argued that this difference should be recognized at the subphylum level, but the lack of ultrastructural proof of syncytial tissues prevented sponge workers from unanimously accepting the proposal (Bergquist 1978). Because hexactinellids are predominantly deep water sponges, specimens were usually obtained by dredging, and consequently were in poor condition. The discovery of shallow-water populations accessible by SCUBA in the late 1970s allowed the first ultrastructural examination of tissues and confirmed that the major tissue component in the sponge constitutes a single giant syncytium (Reiswig 1979; Mackie & Singla 1983).

Advances in our understanding of the physiology (Perez 1996; Wyeth et al. 1996; Leys & Mackie 1997), development (Boury-Esnault & Vacelet 1994; Boury-Esnault et al. 1999), and molecular biology (Koziol et al. 1997; Krüse et al. 1998) of hexactinellids have confirmed the unusual status of this group within the phylum Porifera. However, recent invertebrate texts have yet to acknowledge a proposal for subdivision of sponges into two subphyla (Reiswig & Mackie 1983). Because hexactinellid tissue is unusually difficult to preserve well for either transmission or scanning electron microscopy (TEM, SEM), it is possible that discrepancies in interpretation of preserved tissue by specialists in this field have been the reason why no text carries a clear description of the tissue organization of these sponges. Despite several excellent articles describing fixed tissues from some eight species of hexactinellids (e.g. Mackie & Singla 1983; Reiswig & Mehl 1991; Boury-Esnault & Vacelet 1994), a clear, three-dimensional picture of their tissues is still lacking.

In other sponge groups the flagellated chambers and

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associated tissues, which are called the choanosome, are of considerable importance in determining taxonomic affiliations. The present work uses unconventional techniques to present a three-dimensional perspective of the choanosome, including the incurrent and excurrent water canal system, of two hexactinellids that are found in relatively shallow waters in British Columbia, Canada. The goal of this paper is to provide a clearer understanding of hexactinellid tissue organization in order to confirm and progress beyond the work of Reiswig (1979) and Mackie & Singla (1983).

General organization of hexactinellid sponges

Considering the unusual tissue structure of this group of sponges it is useful to outline the general organization of hexactinellid tissue as known from previous ultrastructural work. An overview of this organization is shown in Fig. 1. Despite differences in spicule skeleton, which is dictyonal (fused) in *Aphrocallistes vastus* and lyssacine (loose) in *Rhabdocalyptus dawsoni,* the general organization of the soft tissues in both sponges is quite similar. Both sponges are basically vase shaped. The body wall is divided into three areas: the dermal epithelium and the atrial epithelium, which by convention are called the dermal and atrial membranes as they are syncytial; the peripheral trabecular network, which lies just within the dermal and atrial membranes; and the choanosome, which contains all the flagellated chambers (Fig. 1B). All of these areas, including the dermal and atrial membranes, are formed by a single, continuous, syncytial tissue called the trabecular reticulum, which is rather like a multinucleate amoeba strung out as a cobweb. The trabecular reticulum is bilayered and encloses a very thin collagenous mesohyl. Thicker strands of the trabecular reticulum have been termed the "cord syncytia" (Reiswig 1979), and probably correspond to bulk cytoplasmic streams in live tissue (Leys 1995). Distinct cells lie within the mesohyl and are connected to each other and to the trabecular reticulum by plugged cytoplasmic bridges (Mackie & Singla 1983). Among these are spherulous cells and thesocytes, which are more common in the peripheral trabecular network, and archaeocytes and choanoblasts, which are found in the choanosome in the walls of the flagellated chambers (Fig. 1C). Sclerocytes, which form the spicule skeleton of the sponge, are multinucleate themselves and are not joined to the trabecular reticulum by plugged junctions (Mackie & Singla 1983).

Methods

Light microscopy

Specimens of *Rhabdocalyptus dawsoni* (LAMBE 1892) were sampled at San Jose Islets, Barkley Sound, British Columbia in February, June, July, August, September, November, and December of 1991 and 1992. Tissue cores of 1.5 cm diameter were taken from the body wall of the sponges using a cork borer. The cores were first placed in plastic vials filled with seawater, which was immediately replaced by Bouin's fixative. At no time were the cores removed from seawater prior to or during fixation. Samples were left in Bouin's for 24 h at 4°C, after which time the cores were rinsed in distilled water and desilicified in 4% hydrofluoric acid overnight, dehydrated in a graded ethanol series, infiltrated with xylene, and embedded in paraffin. Sections of 30 μm thickness were cut from the cores using a rotary microtome, stained with Eriochrome-Cyanin, and viewed with a Leitz Aristoplan compound microscope.

Scanning electron microscopy

Specimens of *R. dawsoni* were collected from 35 m depth at Willis Pt., Saanich Inlet, British Columbia, and at San Jose Islets, Barkley Sound, British Columbia. Specimens of *Aphrocallistes vastus* SCHULZE 1886, were collected from 40 m depth at McCurdy Pt. in Saanich Inlet, British Columbia, Canada.

For plastic replicas, small specimens (≤5 cm long) of *R. dawsoni* were brought to the laboratory at the Bamfield Marine Station, ~30 min after collection at San Jose Islets, Barkley Sound, B.C., placed in a plastic container without removal from sea water, and gently injected from the atrial or dermal side with pigmented liquid plastic (Bateson's #17 Plastic Replica and Corrosion Kit, Polysciences Inc., Warrington, Pa.). Plastic was injected until it became visible at the opposite side of the sponge, and pieces were left in seawater overnight to cure. The sponge tissue was removed by soaking in 30% potassium hydroxide. Remnants of the silica skeleton were dissolved by soaking the casts overnight in 4% hydrofluoric acid. Replicas were broken into pieces with a razor blade, mounted on stubs, coated with gold in an Edwards SB150 sputter coater, and viewed in a Hitachi S-3500 N scanning electron microscope.

For freeze fracture, tissue pieces measuring ~1 cm^3 were cut from the sponge, either immediately after collection from the field or after being maintained in recirculating seawater aquaria for 3 weeks, cleaned of debris (in the case of *R. dawsoni*) and placed in a vial without removal from seawater. The tissue was fixed

in a cocktail fixative consisting of 1% OsO_4, 2% glutaraldehyde, 0.45 M sodium acetate buffer pH 6.4, and 10% sucrose. The fixative was added to the vial containing the piece of sponge in seawater, and the vial was placed on ice. After 30 min to 1 h, the fixative was drawn off to the level of the piece of sponge and fresh fixative was added for an additional 2 h. The pieces were rinsed three times in filtered seawater and dehydrated through 30%, 50%, and 70% ethanol. Pieces were desilicified overnight in 4% hydrofluoric acid in 70% ethanol, then dehydrated further to 100% ethanol and immersed in liquid nitrogen, while still in a glass vial, to fracture the sponge pieces according to the method of Johnston & Hildemann (1982). Fractured pieces were critical-point dried with CO_2, mounted on stubs with silver paint, coated with gold in an Edwards S150B sputter coater, and viewed in a JEOL JSM-35 scanning electron microscope.

Results

Water canal system

In *Rhabdocalyptus dawsoni* large incurrent and excurrent canals alternate all through the body wall (Figs. 1B, 2A). Flagellated chambers occupy the space between the walls of these canals. The incurrent canals are up to 1.25 mm in diameter just below the dermal membrane of the sponge (Fig. 2A), where water enters the sponge through pores, and taper to 0.5 mm toward the atrial membrane, where they become highly branched at the prosopyles of the flagellated chambers (Fig. 2 B, C). Separate incurrent canals feed flagellated chambers near the dermal surface and deeper into the sponge wall (Fig. 2B). Some incurrent canals are connected to each other by branches that cross the middle of the body wall (Fig. 2B,C). Other incurrent canals presumably have direct connections to excurrent canals via branches in the peripheral trabecular region, because the excurrent canals were filled with plastic even though the flagellated chambers had not been filled in those specimens injected from the dermal side (e.g., Fig. 2A). In sponges injected from the atrial side, the plastic did not go further than the flagellated chambers, but in some cases (e.g., Fig. 3B) the incurrent canals were nonetheless filled. Although there was no evidence of accidental rupture of the canals caused by the liquid plastic in any of the replicas examined, this possibility cannot be ruled out. The smallest terminal branches of the replicas of the incurrent canals were flat (Fig. 2D), presumably from where the plastic had filled the spaces between sheets of the trabecular tissue and the prosopyles of the flagellated chambers (e.g., Fig. 3A). At the dermal surface there is a superficial network of canals ~200 μm deep into the sponge (Fig. 2A, C). Another, albeit smaller, network of canals lies against the atrial membrane.

The excurrent canals are ~0.5 mm in diameter at the atrial side of the sponge wall, and taper to 0.25 mm near the dermal edge (Figs. 2A, 3A). The replicas of the excurrent canals were not as branched as those of the incurrent canals, but instead were covered with knobs that represented the flagellated chambers. Some flagellated chambers empty directly into the large (0.25–0.5 mm diameter) canals (Fig. 3A, B), while others vent into smaller, ~300 μm in diameter, branches of the large canals (Fig. 3C).

Although plastic replicas were not made of the canal system in *Aphrocallistes vastus,* scanning electron micrographs of freeze-fractured specimens showed that large channels lead through the body wall to the flagellated chambers. The flagellated chambers vent via canals 50–80 μm in diameter into larger, ~150 μm in diameter, excurrent canals that lead toward the atrial membrane (Fig. 4A). The flagellated chambers closely abut one another and are joined laterally by perforated sheets of the trabecular reticulum.

Flagellated chambers

The chambers have average dimensions of 60 × 45 μm in *A. vastus* and 65 × 30 μm in *R. dawsoni.* In both sponges there is a large apopyle, or exit to the chamber, and in *A. vastus* there is a clear apopylar diaphragm, which limits the diameter at the exit of the chamber to ~20 × 35 μm (Fig. 4B).

The flagellated chambers in both *A. vastus* and *R. dawsoni* are like porous balls suspended by strands of the trabecular reticulum (Fig. 5A). At the chambers the trabecular reticulum is bilayered (Fig. 5B, C). The mesohyl between the two layers is thin in both sponges, thickening only slightly where other strands of the trabecular reticulum join the flagellated chambers. In these thicker areas the mesohyl frequently contains two or three cells, such as choanoblasts or archaeocytes. Choanoblasts are quite round, some 5 μm across, and give rise to enucleate collar bodies that protrude, with a collar of microvilli, through the inner layer of the trabecular reticulum on the inside of the flagellated chambers.

Perforations throughout the bilayered reticulum form prosopyles through which water enters the flagellated chambers (Fig. 6A). In *R. dawsoni* the prosopyles have an average diameter of 4.5 μm (3.5–6.4), while in *A. vastus* the average diameter of the prosopyles is 4.0 μm (3.1–5.0). The inner layer of the trabecular reticulum in the flagellated chamber, termed the primary reticulum (Reiswig 1979), is connected by strands to another layer, the secondary reticulum,

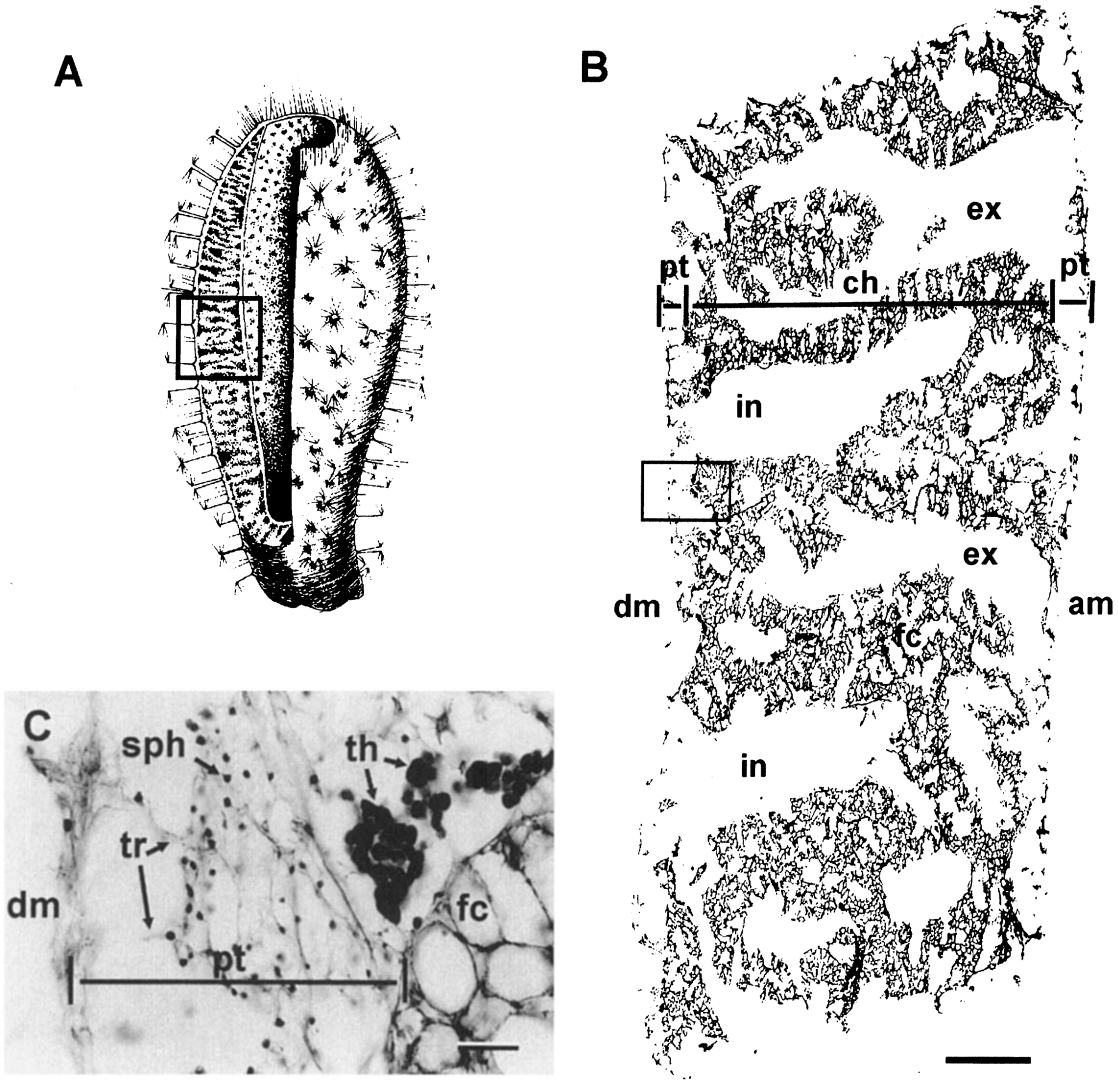


Fig. 1. The general organization of hexactinellid tissue. **A.** A diagram of a rossellid sponge (after Schulze 1887) cut away to show the body wall. The square indicates the portion shown in B. **B.** A transverse section through the body wall of *Rhabdocalyptus dawsoni,* showing the dermal (dm) and atrial (am) membranes, incurrent (in) and excurrent (ex) canals, the peripheral trabecular region (pt), the choanosome (ch), and flagellated chambers (fc). The square indicates the portion shown in C. Composite of photographs of a paraffin section. Scale, 1 mm. **C.** The peripheral trabecular region (pt) at the dermal membrane (dm) of *Rhabdocalyptus dawsoni* showing spherulous cells (sph), thesocytes (th) and flagellated chambers (fc) suspended by strands of the trabecular reticulum (tr). Paraffin section. Scale, 50 μm.

which has perforations that surround the collar of each collar body (Fig. 6A, B). Only in very few instances are the prosopyles and the perforations in the secondary reticulum aligned to allow a clear passage through to the chamber. In most cases the secondary reticulum neatly surrounds each collar so as to prevent any passage of material except through the mesh of the collar microvilli. The space between collar microvilli, measured from scanning electron micrographs, was 0.29 μm (Fig. 6B).

In some flagellated chambers, yet another branch of the trabecular reticulum was found to arise from the secondary reticulum and extend into the centre of the chamber (Fig. 7A). In paraffin sections of specimens of *R. dawsoni* fixed directly upon collection in the field in different months, 1–10% of the flagellated chambers contained a spoke-like inner membrane (Fig 7B). This membrane was no more prevalent in one season than another. However, in specimens of both *R. dawsoni* and *A. vastus* that were kept in recirculating sea water aquaria at the University of Victoria for more than three weeks, an inner membrane was found in all flag-

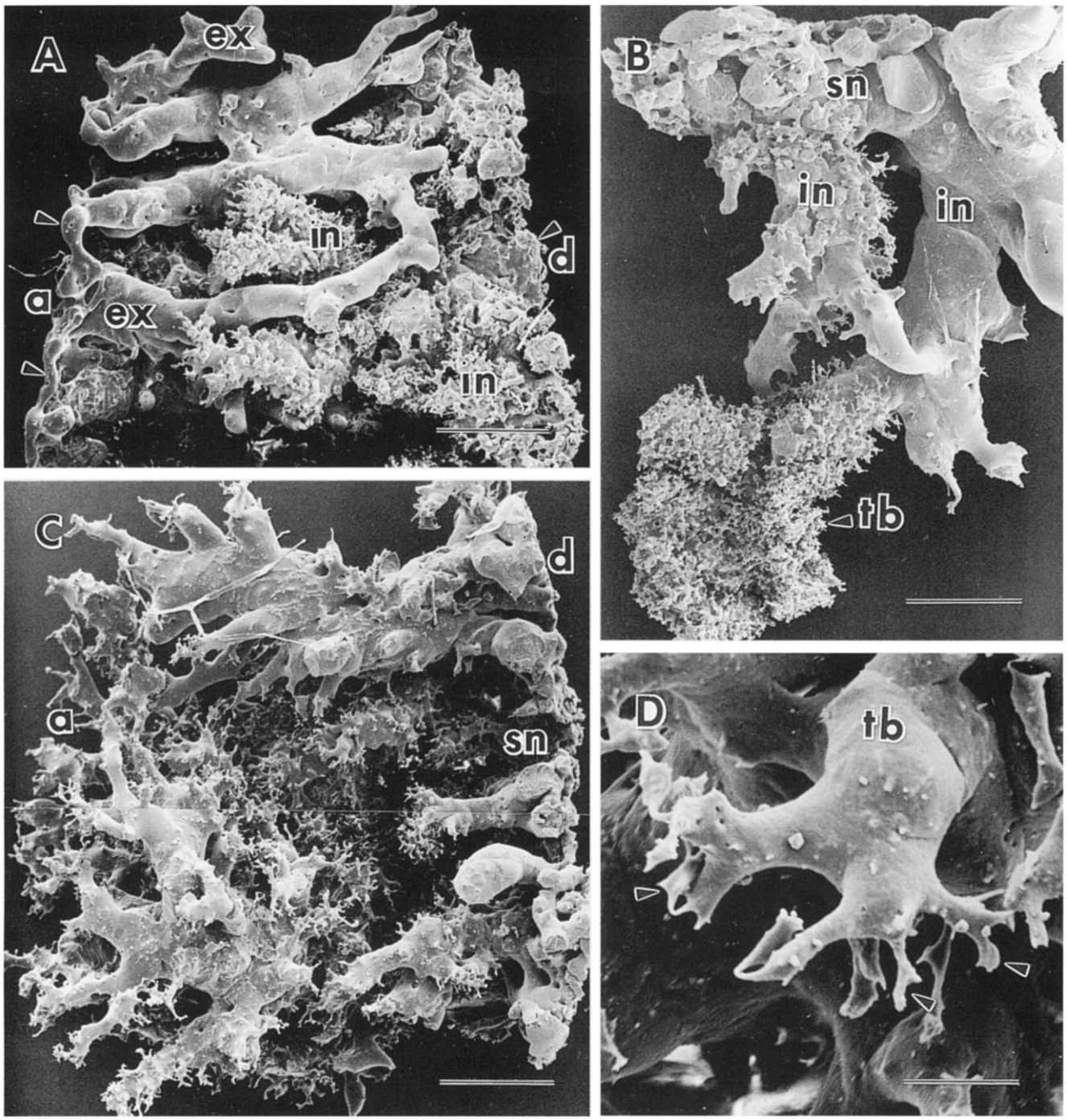


Fig. 2. The water canal system in *Rhabdocalyptus dawsoni.* **A.** A plastic replica of a section of the body wall of *R. dawsoni* showing incurrent (in) and excurrent (ex) canals and the superficial network (arrowheads) on both the dermal (d) and atrial (a) sides. This specimen was filled from the dermal side. Excurrent canals were probably back-filled by plastic coming from direct connections with the incurrent canals because no flagellated chambers are filled (see Fig. 3 and text). Scale, 1 mm. **B.** A replica of two incurrent canals (in) extending from the superficial network (sn); note the fine terminal branches (tb) of the incurrent canals that lie at the back of the flagellated chambers. Scale, 1 mm. **C.** A replica of several incurrent canals leading from the superficial network (sn) at the dermal (d) side of the sponge and becoming highly branched toward the atrial side (a). Scale, 1 mm. **D.** A plastic replica of the terminal branches (tb) of the incurrent canal system. Parts of the terminal branches are flat (arrowheads) where the plastic filled the incurrent spaces at the back of the flagellated chambers. Scale, 100 μm.

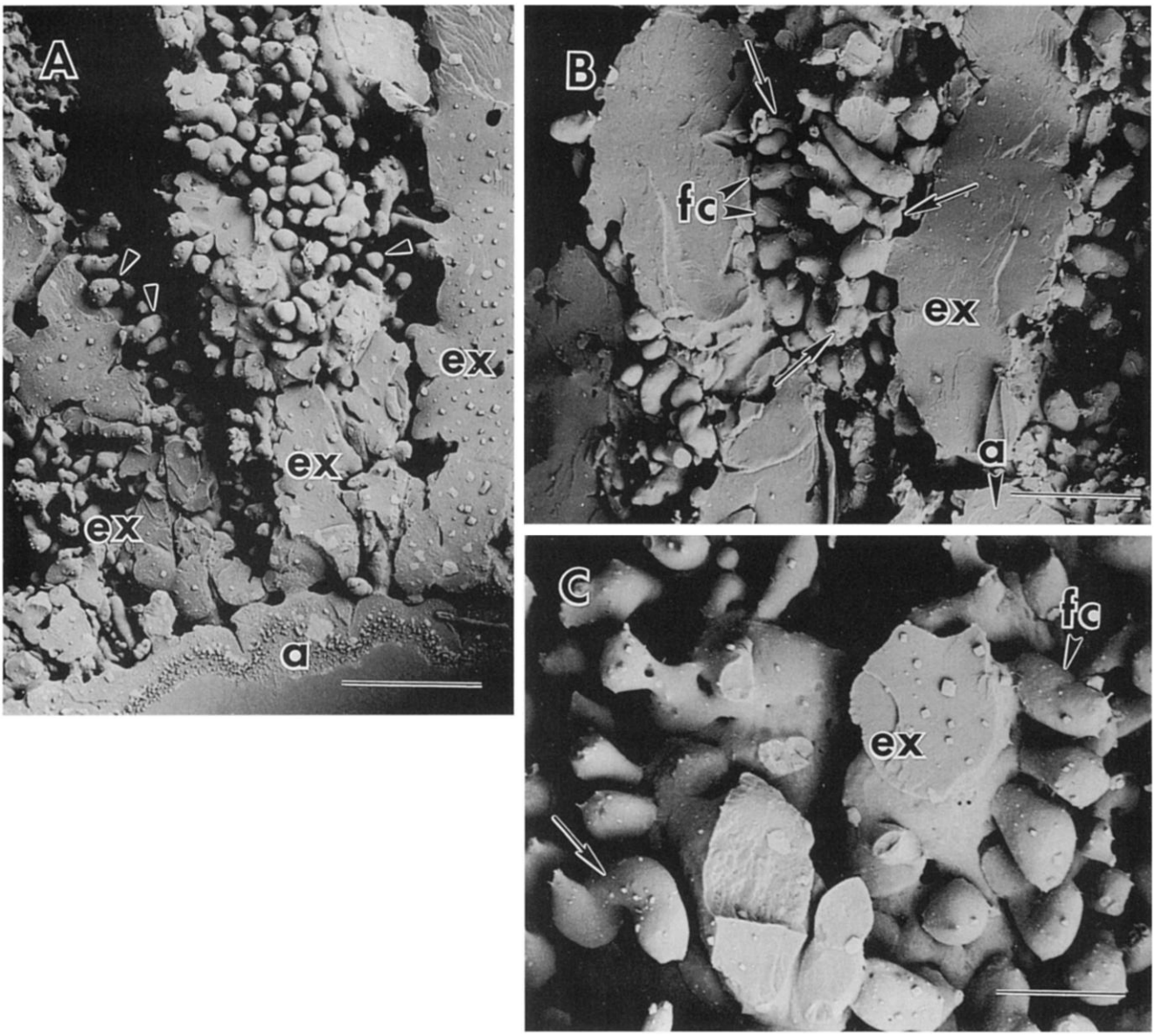


Fig. 3. The excurrent canal system in *Rhabdocalyptus dawsoni.* **A.** A plastic replica filled from the atrial side showing three excurrent canals (ex), each with flagellated chambers (arrowheads) that vent into the atrium (a). Scale, 0.5 mm. **B.** Two excurrent canals (ex) with associated flagellated chambers (fc) that are closely intertwined with branches of the incurrent canals (arrows). The incurrent canals have been filled by plastic entering via connections to the excurrent canals that bypass the flagellated chambers. Atrial side (a); Scale, 300 μm. **C.** Higher magnification of the replicas of flagellated chambers (fc) that vent into branches (ex) of the large excurrent canals. Most chambers do not touch each other (e.g. the three on the right), presumably allowing water flow to access all sides; however, a few closely abut one another (arrow). The plastic has not passed through the prosopyles in the flagellated chambers into the incurrent canals, and in this specimen the incurrent canals are not filled by connections that bypass the flagellated chambers. Scale, 100 μm.

ellated chambers and was so pronounced that it compartmentalized each collar body in the flagellated chambers (Fig. 7C, D).

The choanosome showed a seasonal change in composition. In specimens of *R. dawsoni* collected June–September the connections between flagellated chambers were filled with groups, or 'congeries', of archaeocytes, the embryos of hexactinellids. In sections of sponges collected in June these were 60 μm in diameter, approximately the size of flagellated chambers, and in pieces collected in September many were as large as 100 × 60 μm. Very few archaeocyte

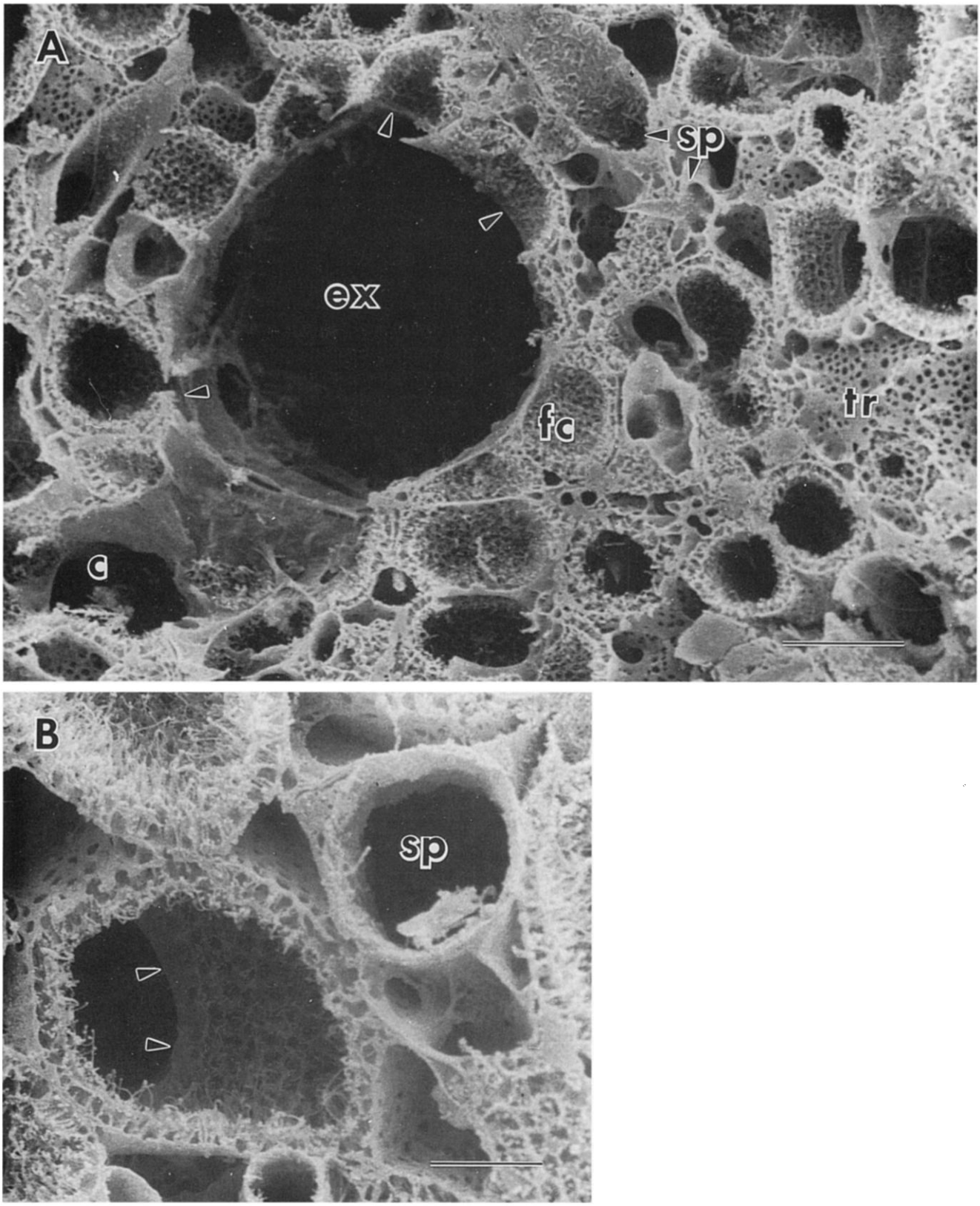
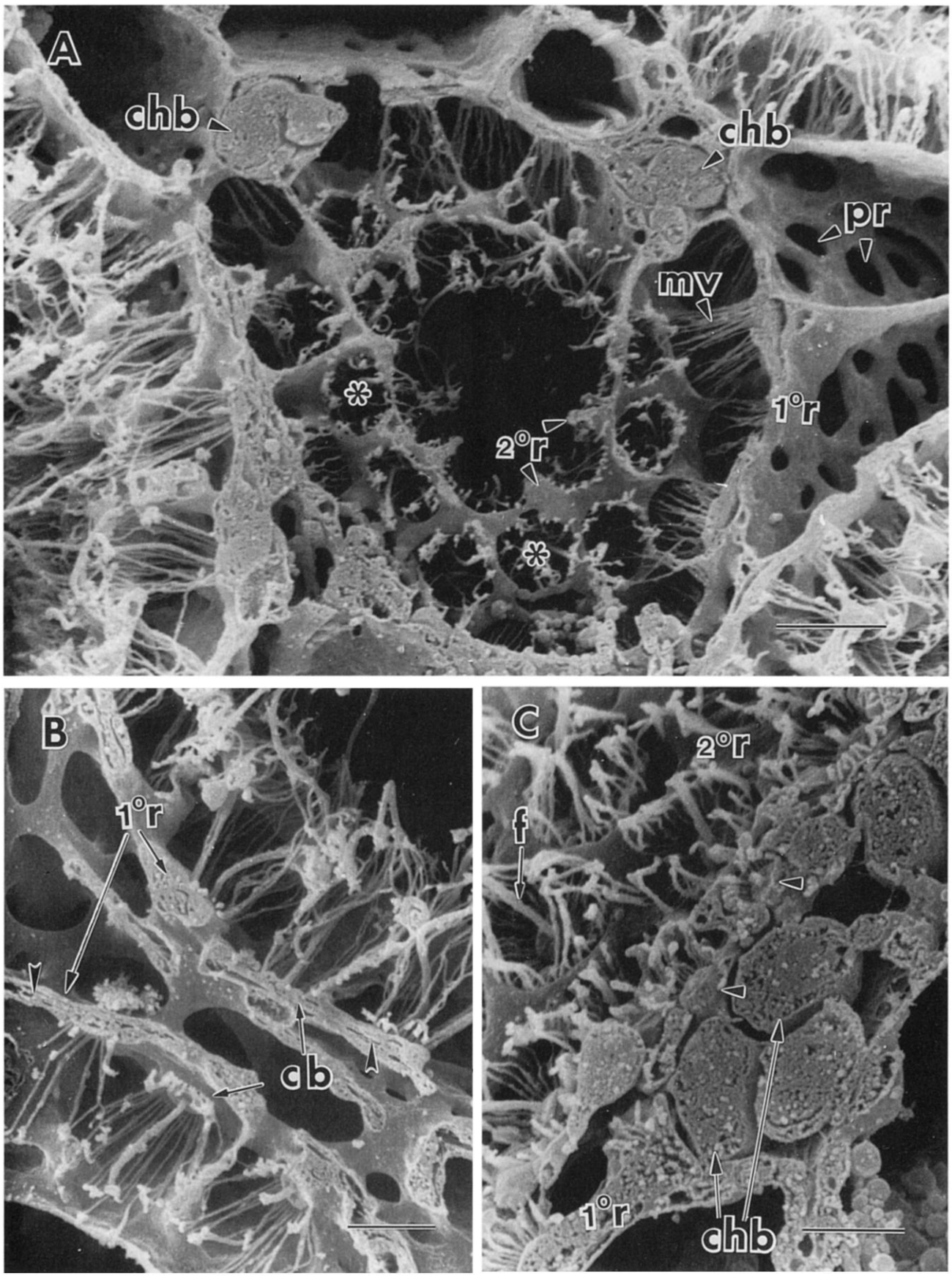


Fig. 4. The choanosome of *Aphrocallistes vastus* (SEM). **A.** A fracture parallel to the dermal membrane showing numerous flagellated chambers (fc), a large excurrent canal (ex), and spaces left by dissolved spicules (sp). Some chambers exit directly into the large central canal (arrowheads). Scale, 50 μm. **B.** The apopylar diaphragm (arrowheads) of a flagellated chamber from *A. vastus.* (Spicule spaces, sp). Scale, 20 μm.



congeries were found in pieces of sponge tissue collected during winter months.

Discussion

Hexactinellid sponges may reach 1m in length (Dayton et al. 1974; Leys & Lauzon 1998) and, as such, probably have the largest single, continuous, syncytial tissue known in the Metazoa. Despite two formal proposals to reclassify hexactinellids as a subphylum of the Porifera based on their syncytial tissues (Reid 1963; Reiswig & Mackie 1983), recent texts continue to describe hexactinellids as one of three classes of sponges. One reason for this may be that hexactinellids are difficult to preserve well for ultrastructural studies, and consequently the combination of cellular and syncytial tissues is difficult to comprehend. The present study provides a series of three-dimensional images of hexactinellid tissue, which aim to clarify our understanding of the syncytial and cellular organization of this group. This paper also presents new information on the orientation of flagellated chambers with respect to incurrent and excurrent canals, the presence of reproductive elements in the choanosome, and the formation of an inner membrane in hexactinellid flagellated chambers.

Choanosome

All species of hexactinellid studied to date have large flagellated chambers that vent into excurrent canals, which exit at the atrial membrane (Schulze 1880, 1887, 1899; Ijima 1901, 1904; Reiswig 1979; Boury-Esnault & De Vos 1988; Reiswig 1991; Reiswig & Mehl 1991, 1994; Boury-Esnault & Vacelet 1994). Simpson (1984) tentatively called the hexactinellid canal system syconoid, but from the information derived from plastic replicas here, it is clear that there are channels for collecting the exhalant water prior to venting it into the atrium, typical of a leuconoid construction. Although recent ultrastructural investigations of hexactinellid tissue do not show the presence of incurrent canals, both Schulze (1887) and Ijima (1901) clearly described well-defined incurrent and excurrent canals in these sponges, also shown by the plastic replicas here. In fact their descriptions of soft tissues and of the water canal system in hexactinellids were remarkably accurate, given the level of resolution available to microscopists at the time.

The plastic replicas were very informative about the canal system in *Rhabdocalyptus dawsoni.* At the dermal surface there is a network of interconnecting canals, that corresponds to the superficial network in *Petrosia ficiformis* (Bavastrello et al. 1988) and which occupies the space termed the peripheral trabecular region in *Farrea occa* (Reiswig & Mehl 1991), and the subdermal trabecular net in *Euplectella marshalli* (Schulze 1887). The incurrent canals are large and feed large areas of flagellated chambers, as seen by the highly branched endings of the replicas of the incurrent canals. As described by Schulze (1887), some inhalant canals lead directly to groups of flagellated chambers located deeper into the sponge body wall. Others feed flagellated chambers nearer the dermal surface.

Exhalent canals are large and have finger-like branches into which the flagellated chambers empty. The orientation of the flagellated chambers was clearly seen in plastic replicas that were injected from the atrial side, and is remarkably similar to that described for *E. marshalli* (Ijima 1901), and other rossellid sponges (Ijima 1904). The prosopyles and the perforations in the secondary reticulum are apparently too small (~5 μm in diameter) to allow the liquid plastic through as no plastic was found past the flagellated chambers in specimens injected from the atrial side, except where it had bypassed the flagellated chambers by other routes. Moreover, no plastic was found in the flagellated chambers in specimens injected from the dermal side, although plastic had filled the excurrent canals. There was no evidence that the canal walls had been ruptured during injection of the liquid plastic. The smallest terminal branches of the incurrent canals, which presumably would be under higher pressure

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Fig. 5. Details of the flagellated chambers of *A. vastus* and *R. dawsoni* (SEM). **A.** A fracture through the back of a flagellated chamber of *A. vastus* showing the primary reticulum (1°r) with the prosopyles (pr), through which water enters the chamber, and perforations (asterisks) in the secondary reticulum (2°r) that surrounds the microvilli (mv) of each collar. Where strands of the trabecular reticulum join the flagellated chamber, the mesohyl is thicker and encloses choanoblasts (chb). Scale, 5 μm. **B.** A fracture through the wall of two adjoining flagellated chambers from *A. vastus* shows that the primary reticulum (1°r) of each chamber partially encloses collar bodies (cb) in a thin collagenous mesohyl (arrowheads). Scale, 3 μm. **C.** A fracture through the edge of a flagellated chamber from *R. dawsoni* shows several choanoblasts (chb) with their respective collar bodies (arrowheads) in the mesohyl of the primary reticulum (1°r). The flagella (f) and collar of microvilli can be seen projecting through the secondary reticulum (2°r) on the inside of the flagellated chamber. Scale, 3 μm.

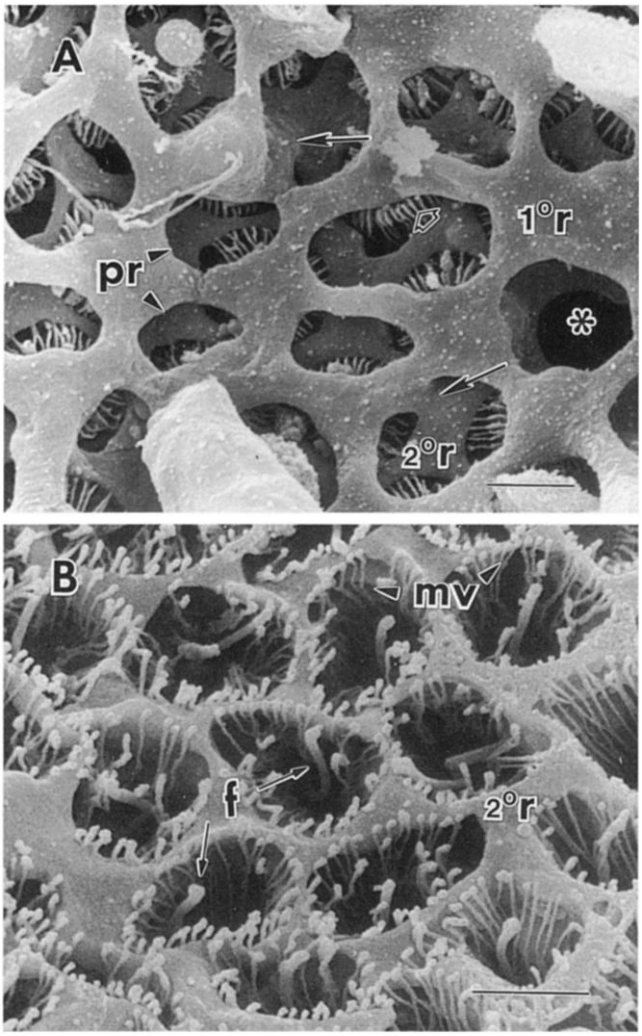

Fig. 6. The primary and secondary reticula (SEM). **A.** A view from the back of a flagellated chamber in *R. dawsoni* shows perforations in the primary reticulum (1°r) that represent the prosopyles (pr). The collar microvilli (open arrow) arising from collar bodies that lie in the primary reticulum, can be seen projecting through the secondary reticulum (2°r). Strands of the primary reticulum connect to the secondary reticulum (arrows). In a few places the perforations in the secondary reticulum are directly opposite the prosopyles (asterisk) allowing a direct passage to the inside of the chamber. Scale, 3 μm. **B.** A fracture across the collar microvilli (mv) and flagella (f) on the inside of a flagellated chamber of *A. vastus* shows that the secondary reticulum (2°r) completely surrounds each of the collars. Scale, 3 μm.

than the larger canals, were always intact. The canals in the subdermal trabecular network did not appear to have been ruptured, as one would expect the liquid plastic to have penetrated the flagellated chambers as when injected from the atrial side. Two possibilities remain. The canals in the sub-'atrial' trabecular network may have ruptured from the pressure of injecting the sponges from the dermal side, allowing plastic to enter the excurrent canals. Alternatively, some of the sub-'atrial' incurrent canals have direct connections to excurrent canals, which suggests that passive water flow may allow water to bypass the flagellated chambers, as in the demosponge *P. ficiformis* (Bavastrello et al. 1988). Neither possibility can be conclusively confirmed or refuted.

Although Reiswig & Mehl (1991) found no apopylar diaphragm in the flagellated chambers of *F. occa,* there is a distinct apopylar diaphragm in the flagellated chambers in *Aphrocallistes vastus.* It is possible with improved fixation techniques that a diaphragm would be found at the exit to flagellated chambers in other hexactinellids.

Secondary reticulum

The secondary reticulum has been found in all but two hexactinellids examined so far and is considered to be a characteristic of these sponges (Mackie & Singla 1983). Its absence in *Dactylocalyx* (Reiswig 1991) and *Caulophacus cyanae* (Boury-Esnault & De Vos 1988) was attributed to possible poor fixation of specimens. It has been suggested that the secondary reticulum forms a support for the collar microvilli and prevents back flow of water (Reiswig 1979). However, in the three-dimensional images presented here, it can be seen that this layer of the trabecular reticulum forms an almost complete barrier to the direct passage of water into the flagellated chambers. As noted by Reiswig & Mehl (1991), the hexactinellid secondary reticulum is very similar to the barrier formed by central cells that fit snugly around the collars of choanocytes in the demosponge *Pellina fistulosa* (Langenbruch & Jones 1989) and to a glycocalyx mesh that occupies the space between the tips of collars in freshwater sponges (Weissenfels 1992). Both are suggested to increase the efficiency of feeding by preventing particles from passing around the collars. Likewise, particles too large to pass through the collar microvilli mesh in hexactinellids would most probably come into contact with the secondary reticulum as the water is drawn into the flagellated chambers. Furthermore, the images of freeze-fractured specimens showed that the primary reticulum completely envelops the collar bodies, preventing their direct exposure to the incurrent flow. These observations support the results of Perez (1996) and Wyeth (1999) that indicate the primary and secondary reticula are the main feeding structures in these sponges.

Inner membrane

The presence of a structure referred to as an "inner membrane" by Reiswig & Mehl (1991) was confirmed

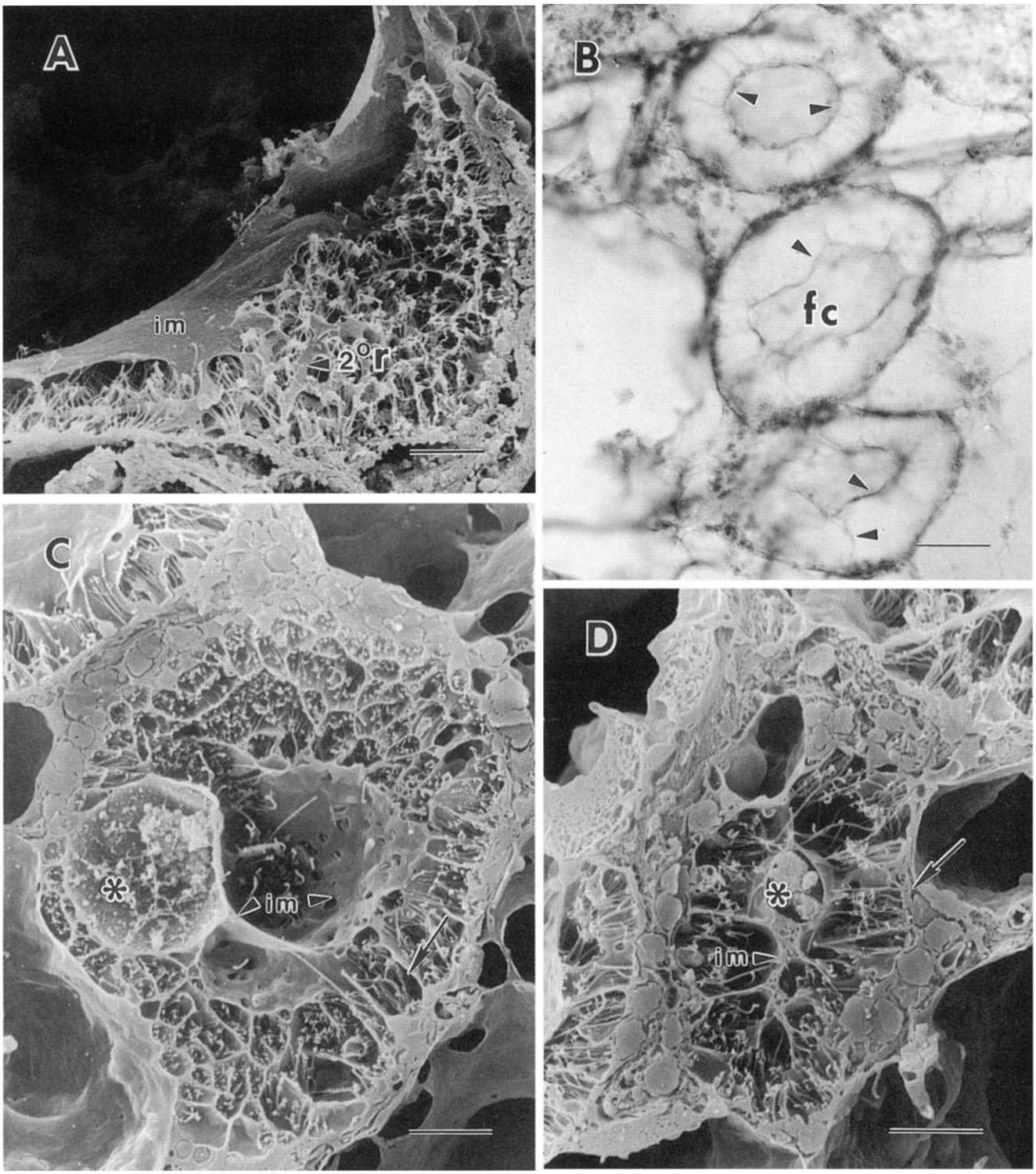

Fig. 7. The inner membrane. **A.** In a flagellated chamber from *A. vastus,* a sheet-like membrane (im) stretches across the tips of some of the flagella and reaches down to join with the secondary reticulum (2°r) (SEM). Scale, 10 μm. **B.** Inner membranes (arrowheads) in flagellated chambers (fc) of paraffin sections from pieces of *R. dawsoni* that were fixed directly after collection in the field (light microscopy). Scale, 20 μm. **C** and **D.** Fractured chambers from pieces of *R. dawsoni* that were kept in laboratory aquaria for three weeks (SEM). The inner membrane (im) has compartmentalized all of the collars in the flagellated chambers and forms a sheet overlying the tips of the flagella with large, debris-filled phagosomes (asterisk). Scale, 10 μm.

here for both *R. dawsoni* and *A. vastus*. Since inner membranes were found in up to 10% of the flagellated chambers in pieces from *R. dawsoni* individuals that were fixed at different months throughout the year, and were no more prevalent at any particular month, it is unlikely that they reflect a seasonal deterioration of the tissue.

Reiswig & Mehl (1991) compared the inner membrane in *F. occa* with that of central cells from other sponges. Central cells have been found in 5–15 % of the flagellated chambers of many species of Demospongiae, Calcarea, and Sclerospongiae. They have been speculated to stop water flow by arresting the flagella, to phagocytose debris, to produce the glycocalyx adhesive substance for the collars, or to simply be wandering mesenchymal cells (e.g., Duboscq & Tuzet 1939; Pavans de Ceccatty 1955; Connes et al. 1971; Reiswig & Brown 1977), but it is difficult to come by concrete evidence of their function. Considering that hexactinellids are capable of stopping their feeding current almost instantly upon mechanical stimulation (Lawn et al. 1981; Mackie et al. 1983; Leys & Mackie 1997), it seems unlikely that the development of an inner membrane would be sufficient to stop the flow, as they occur in only 10% of flagellated chambers. The evidence presented here, that sponges kept in recirculating-seawater aquaria develop inner membranes in all of the flagellated chambers, suggests that the inner membranes are ameboid extensions of the trabecular reticulum that absorb non-functioning flagellated chambers. Although in their study of *R. dawsoni* Mackie & Singla (1983) found no evidence that the collar bodies were absorbed, it is possible that in their sectioning they did not encounter any of the 10% of chambers that might have been absorbed. Wyeth (1999) found many examples of collar microvilli in membrane-bound compartments within the trabecular reticulum of sandwich cultures prepared from regenerating fragments of *R. dawsoni,* which confirms the ability of the trabecular reticulum to absorb collar bodies.

Seasonal reproduction

Examination of the tissue from pieces of *R. dawsoni* that were collected at different months throughout the year showed the seasonal appearance of archaeocyte congeries. Clumps of archaeocytes as large as flagellated chambers were found in the mesohyl at the edges of chambers and at thickenings in strands of the trabecular reticulum in sponge pieces collected in June, July, August, and September. In September some large parenchymella-like objects were found in the excurrent spaces, but their identification as either embryos or larvae was inconclusive as no live larvae were encountered.

Reports on the larvae of hexactinellids indicate that these sponges are reproductive year-round (Okada 1928; Boury-Esnault & Vacelet 1994). Okada suggested that in the deep-sea environment hexactinellids might not experience seasons, which would otherwise influence their reproductive period. However, Marliave (1992) reported finding juveniles (<1cm) of *R. dawsoni* predominantly in autumn months. Furthermore, in his description of the choanosome of *Rhabdocalyptus victor,* Ijima (1904) noted that specimens that lacked conspicuous congeries of archaeocytes were immature, which suggests that at least some deep-sea species may also have seasonal reproductive periods.

Syncytial vs. cellular tissues

Studies of the ultrastructure of fixed tissue from *R. dawsoni* and *A. vastus* (Reiswig 1979; Pavans de Ceccatty 1982; Mackie & Singla 1983), of the cytoskeletal architecture of aggregates from both sponges, and of live tissue both as aggregates and as sandwich cultures (Leys 1995, 1998), have definitively shown that the majority of the tissue in these sponges consists of a giant, multinucleated syncytium.

The scanning electron micrographs of fractured tissue from *R. dawsoni* and *A. vastus* show the choanosome of both sponges to be similar to that of *F. occa* (Reiswig & Mehl 1991) and *Oopsacas minuta* (Boury-Esnault & Vacelet 1994). The trabecular reticulum (= trabecular syncytium) extends from the dermal membrane to the atrial membrane through the whole sponge, and surrounds the cellular elements, which include archaeocytes, spherulous cells, thesocytes, and choanoblasts. At the flagellated chambers the trabecular reticulum forms two branches, the primary and secondary reticula (Fig. 8). The former is a bilayered sheet that encloses a thin collagenous mesohyl which contains choanoblasts and collar bodies. The cytoplasmic connection via perforated plugged junctions between the trabecular reticulum and cells, including choanoblasts with their collar bodies, has been shown by transmission electron microscopy (Mackie 1981; Pavans de Ceccatty & Mackie 1982; Mackie & Singla 1983). Although the collar bodies and choanoblasts were previously known collectively as a "choanosyncytium" (Reiswig 1979; Mackie & Singla 1983), it is clear here that choanoblasts—like archaeocytes, spherulous cells, and thesocytes—are uninucleate when completely formed and should be considered to be cellular, while the collar bodies are enucleate outgrowths of those cells.

The physiological implications of a single continu-

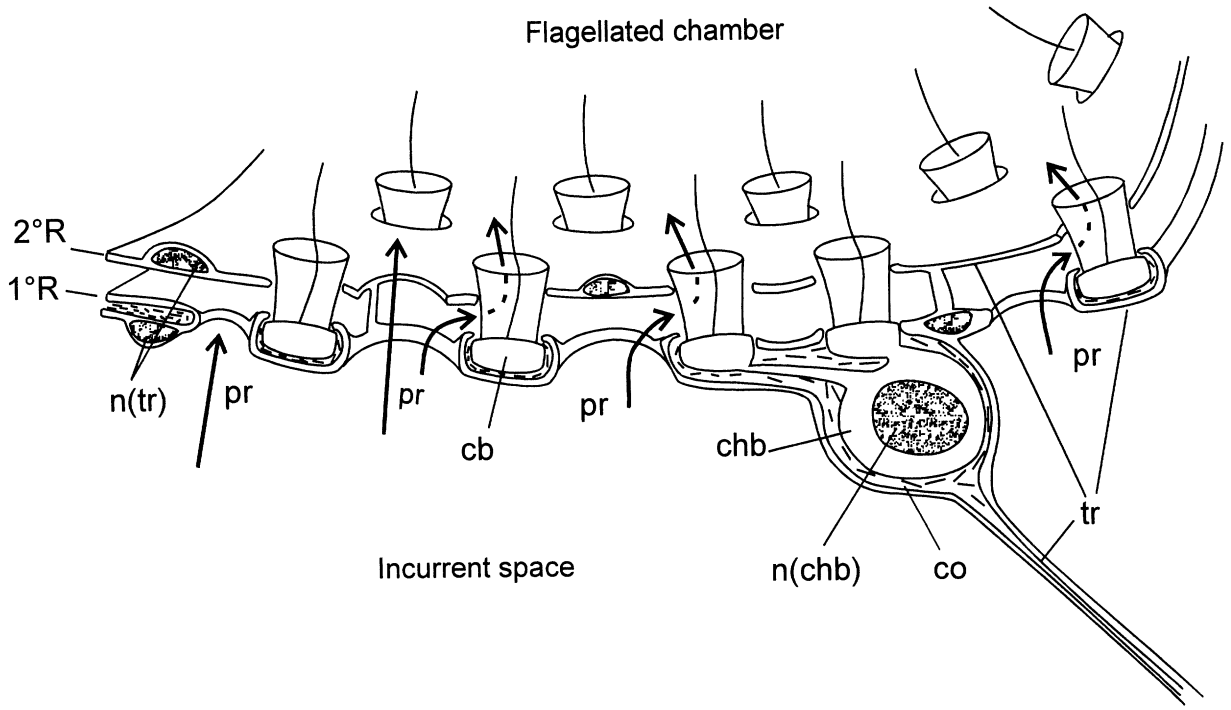

Fig. 8. A schematic drawing of a cross section of a flagellated chamber in hexactinellids. The primary reticulum (1°R), a branch of the syncytial trabecular reticulum (tr), surrounds the cellular choanoblasts (chb) and their enucleate collar bodies (cb) in a thin collagenous mesohyl (co). The secondary reticulum (2°R) is another branch of the trabecular reticulum that forms a single layer around the collars of the collar bodies. Water is drawn through the prosopyles (pr) to the filtering mesh of the collar microvilli; there are only a few direct routes through to the chamber (indicated by the arrows). n(tr): nuclei of the trabecular reticulum; n(chb): nucleus of a choanoblast.

ous network of membrane-bound cytoplasm in these sponges are profound. Stimuli to the dermal surface of hexactinellids have been shown to generate electrical impulses that turn off the feeding current, presumably by flagellar arrest (Leys & Mackie 1997). Since there is no clear evidence for gap junctions in sponges (see Pavans de Ceccatty et al. 1970; Green & Bergquist 1982), the only way that an electrical impulse can pass unimpeded through hexactinellid tissue is via the trabecular reticulum which reaches all parts of the flagellated chambers. At the flagellated chambers, the signal presumably passes to each collar body through the plugged junction, as there is no membrane barrier (Mackie et al. 1983). With respect to feeding, nutrients taken in at the flagellated chambers (Wyeth et al. 1996) may be transported intrasyncytially to all parts of the tissue, rather than by the amoeboid movement of cells as in cellular sponges (Imsiecke 1993; Leys & Reiswig 1998).

It remains to be learned how the syncytial trabecular reticulum forms. Recent evidence from embryos of the hexactinellid *O. minuta* (Boury-Esnault 1999) suggests that the cells are connected by plugged cytoplasmic bridges in early embryos, and that the synctyial tissue arises from particular cells during larval development. Current work is focusing on how new tissue for the trabecular reticulum forms.

Acknowledgments. I thank the director and staff of the Bamfield Marine Station for use of their facilities for portions this work, N. Lauzon and J. Cosgrove for help collecting the sponges, and L. Sun for assistance with the paraffin sectioning. R.C. Wyeth, L.R. Page, and G.O. Mackie gave helpful comments on earlier versions of this manuscript. This research was supported by a Natural Science and Engineering Research Council (NSERC) grant (OGPOO1247) to G.O.M., and an NSERC postgraduate scholarship to the author.

References

Bavestrello G, Burlando B, & Sara M 1988. The architecture of the canal systems of *Petrosia ficiformis* and *Chondrosia reniformis* studied by corrosion casts (Porifera, Demospongiae). Zoomorphology 108: 161–166.

Bergquist PR 1978. Sponges. Hutchinson and Co., London. 268pp.

Bidder GP 1929. Sponges. Encyclopaedia Britannica 14th Edition. 254–261.

Boury-Esnault N, Efremova S, Bézac C, & Vacelet J 1999.

Reproduction of a hexatinellid sponge: first description of gastrulation by cellular determination in the Porifera. Invertebrate Reproduction and Development (in press).

Boury-Esnault N & De Vos L 1988. *Caulophacus cyanae,* n. sp., une éponge hexactinellide des sources hydrothermales. Biogéographie du genre *Caulophacus* Schulze, 1887. Oceanologica Acta 8: 51–60.

Boury-Esnault N & Vacelet J 1994. Preliminary studies on the organization and development of a hexactinellid sponge from a Mediterranean cave, *Oopsacas minuta.* In: Sponges in Time and Space. van Soest RWM, van Kempen TMG, & Braekman J, eds., pp. 407–416. AA Balkema, Rotterdam.

Connes R, Diaz JP, & Paris J 1971. Choanocytes et cellule centrale chez la démosponge *Suberites massa* Nardo. C.R. Acad. Sc. Paris, 273: 1590–1593.

Dayton PK, Robilliard GA, Paine RT, & Dayton LB 1974. Biological accommodation in the benthic community at McMurdo Sound, Antartica. Ecological Monographs 44: 105–128.

Duboscq O & Tuzet O 1939. Les diverses formes des choanocytes des éponges calcaires hétérocoeles et leur signification. Arch. Zool. Exp. Gen. 80: 353–388.

Green CR & Bergquist PR 1982. Phylogenetic relationships within the invertebrata in relation to the structure of septate junctions and the development of occluding junctional types. J. Cell Sci. 53: 270–305.

Ijima I 1901. Studies on the Hexactinellida. Contribution I (Euplectellidae). J. Coll. Sci. imp. Univ. Tokyo 15: 1–299.

——— 1904. Studies on the Hexactinellida. Contribution IV. (Rossellidae). J. Coll. Sci. imp. Univ. Tokyo 28: 13–307.

Imsiecke G 1993. Ingestion, digestion, and egestion in *Spongilla lacustris* (Porifera, Spongillidae) after pulse feeding with *Chlamydomonas reinhardtii.* Zoomorphology 113: 233–244.

Johnston IS & Hildemann WH 1982. Cellular organization in the marine demosponge *Callyspongia diffusa.* Mar. Biol. 67: 1–7.

Koziol C, Leys SP, Müller IM, & Müller WEG 1997. Cloning of Hsp70 genes from the marine sponges *Sycon raphanus* (Calcarea) and *Rhabdocalyptus dawsoni* (Hexactinellida). An approach to solve the phylogeny of sponges. Biol. J. Linn. Soc. 62: 581–592.

Kruse M, Leys SP, Müller IM, Müller WEG 1998. Phylogenetic position of the Hexactinellida within the phylum Porifera based on amino acid sequence of the protein kinase C from *Rhabdocalyptus dawsoni.* J. Mol. Evol. 46: 721–728.

Langenbruch P & Jones WC 1989. A new type of central cell in the choanocyte chambers of *Pellina fistulosa* (Porifera, Demospongiae). Zoomorphology 109: 11–14.

Lawn ID, Mackie GO, & Silver G 1981. Conduction system in a sponge. Science 211: 1169–1171.

Leys SP 1995. Cytoskeletal architecture and organelle transport in giant syncytia formed by fusion of hexactinellid sponge tissues. Biol. Bull. 188: 241–254.

——— 1998. Fusion and cytoplasmic streaming are characteristics of at least two hexactinellids: examination of cultured tissue from *Aphrocallistes vastus.* In: Sponge Sciences—Multidisciplinary Perspectives. Watanabe Y & Fusetani N, eds., pp. 215–226. Springer-Verlag, Tokyo.

Leys SP & Mackie GO 1997. Electrical recording from a glass sponge. Nature 387: 29–31.

Leys SP & Lauzon RJN 1998. Hexactinellid sponge ecology: growth rates and seasonality in deep water sponges. J. Exp. Mar. Biol. Ecol. 230:111–129.

Leys SP & Reiswig HM 1998. Nutrient transport pathways in the neotropical sponge *Aplysina.* Biol. Bull. 195:30–42.

Mackie GO 1981. Plugged syncytial interconnections in hexactinellid sponges. J. Cell Biol. 91: 103a.

Mackie GO & Singla CL 1983. Studies on hexactinellid sponges. I Histology of *Rhabdocalyptus dawsoni* (Lambe, 1873). Phil. Trans. R. Soc. Lond. B 301: 365–400.

Mackie GO, Lawn ID, & Pavans de Ceccatty M 1983. Studies on hexactinellid sponges. II. Excitability, conduction and coordination of responses in *Rhabdocalyptus dawsoni* (Lambe 1873). Phil. Trans. R. Soc. Lond. B 301: 401–418.

Marliave JB 1992. Environmental monitoring through natural history research. Can. Tech. Rep. Fish Aquat. Sci. 1879: 199–209.

Okada Y 1928. On the development of a hexactinellid sponge, *Farrea sollasii.* J. Fac. Sci. imp. Univ. Tokyo 4: 1–29.

Pavans de Ceccatty M 1955. Le systeme nerveux des éponges calcaires et siliceuses. Ann. Sci. Nat. Zool. Biol. Anim. 17: 203–288.

——— 1982. In vitro aggregation of syncytia and cells of a hexactinellida sponge. Dev. Comp. Immunol. 6: 15–22.

Pavans de Ceccatty M, Thiney Y, & Garrone R 1970. Les bases ultrastructurales des communications intercellulaires dans les oscules de quelques éponges. Symp. Zool. Soc. Lond. 25:449–466.

Pavans de Ceccatty M, & Mackie G 1982. Genèse et évolution des interconnexions syncytiales et cellulaires chez une éponge Hexactinellide en cours de réagrégation après dissociation *in vitro.* C.R. Acad. Sc. Paris, t.294: 939–944.

Perez T 1996. La rétention de particules par une éponge hexactinellide, *Oopsacas minuta* (Leucopsacasidae): le rôle du réticulum. C. R. Acad. Sci. Paris, Sciences de la vie 319: 385–391.

Reid REH 1963. Hexactinellida or Hyalospongea. J. Paleontology 37: 232–243.

Reiswig HM 1979. Histology of hexactinellida (Porifera). Colloques Int. Cent. Natn. Rech. scient. 291: 173–180.

——— 1991. New perspectives on the hexactinellid genus *Dactylocalyx* Stuchbury. In: Fossil and recent sponges. Reitner J & Keupp H, eds., pp. 7–20. Springer-Verlag, Berlin.

Reiswig HM & Brown MJ 1977. The central cells of sponges. Zoomorphologie 88: 81–94.

Reiswig HM & Mackie GO 1983. Studies on hexactinellid sponges III. The taxonomic status of Hexactinellida within the Porifera. Phil. Trans. R. Soc. Lond. B 301: 419–428.

Reiswig HM & Mehl D 1991. Tissue organization of *Farrea occa* (Porifera, Hexactinellida). Zoomorphology 110: 301–311.

——— 1994. Reevaluation of *Chonelasma* (Euretidae) and *Leptophragmella* (Craticulariidae) (Hexactinellida). In: Sponges in Time and Space. van Soest RWM, van Kempen TMG, & Braekman J-C, eds., pp. 151–165. A.A. Balkema, Rotterdam.

Schulze FE 1880. On the structure and arrangement of the soft parts in *Euplectella aspergillum.* Roy. Soc. Edinb. Trans. 29: 661–673.

——— 1887. Report on Hexactinellida. Challenger Report 21: 1–513.

——— 1899. Zur Histologie der Hexactinelliden. Sber.dt. Akad. Wiss. 14: 198–209.

Simpson TL 1984. The cell biology of sponges. Springer Verlag, New York. 662 pp.

Weissenfels N 1992. The filtration apparatus for food collection in freshwater sponges (Porifera, Spongillidae). Zoomorphology 112: 51–55.

Wyeth RC 1999. Video and electron microscopy of particle feeding in sandwich cultures of the hexactinellid sponge, *Rhabdocalyptus dawsoni.* Invert. Biol. 118(3): 236–242.

Wyeth RC, Leys SP, & Mackie GO 1996. Use of sandwich cultures for the study of feeding in the hexactinellid sponge *Rhabdocalyptus dawsoni* (Lambe, 1892). Acta Zool. 77: 227–232.